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EFFECT OF REPETITIVE EXPOSURE TO GAMMA RAYS ON THE HEMATOPOIETIC SYSTEM OF THE RAT

Research and Development Technical Report USNRDL-TR-12
NM 006 015

27 August 1954

by

S. J. Baum
D. J. Kimeldorf
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DEPARTMENT OF THE NAVY ★ NAVAL RADIOLOGICAL DEFENSE LABORATORY ★

U.S. NAVAL RADIOLOGICAL DEFENSE LABORATORY

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Health and Biology

Technical Objective
AW-6

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ABSTRACT

Male rats were subjected to a series of one or more doses of 75 r gamma irradiation at seven day intervals between exposures. Hematological examinations were made following the last exposure in each series of irradiations. The maximum changes observed were, to a great extent, independent of the number of previous exposures, and appeared to reflect principally the effects of the last irradiation. These results were ascribed to the regenerative capacity of the hematopoietic system to recover during the seven day interval between radiation exposures. A discussion of these results and their correlation with the findings of other experiments is given.

SUMMARY

The Problem

To determine the effect of periodic exposure to low intensity gamma radiation upon hematopoiesis in the rat.

Findings

Rats were subjected to a series of one or more doses of 75 r gamma irradiation, at seven day intervals between exposures. Hematological examinations were made following the last exposure in each series of irradiations. The maximum changes observed were, to a great extent, independent of the number of previous exposures, and appeared to reflect principally the effects of the last irradiation. These results were ascribed to the regenerative capacity of the hematopoietic system to recover during the seven day interval between radiation exposures.

ADMINISTRATIVE INFORMATION

This work was part of Project NM 006-015.04, Phase 12, Technical Objective AW-6.

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REPORT OF INVESTIGATION

The hematological effects of acute ionizing radiations have been described extensively in recent review articles.[1,2,4] The effects of prolonged exposure to low intensity radiations and the effects of dose fractionation or periodic radiation exposures are somewhat more difficult to interpret because of the complex interaction of destructive and regenerative factors involved.

When rats were exposed daily to low (7.7 r) doses of X rays relatively few changes were observed in the number of circulating blood cells, although the exposure was continued for 250 days.[5] Mice subjected to 1.1, 4.4 or 8.8 r of gamma rays during eight hours daily[6] for a one year period exhibited no significant erythrocyte changes. However, daily doses of 33.5 r of X rays[5] have been demonstrated to be sufficient to cause a slight but distinct decrease in erythrocyte counts in rats. The dose-response relationship is somewhat species dependent. For example, a significant decrease in the erythrocyte estimate in guinea pigs exposed to 8.8 r gamma radiation for eight hours daily was observed by Lorenz, et al.[7]

Leukocytes are much more sensitive to ionizing radiation. Daily exposures to 7.7 r X irradiation caused a distinct decrease in the number of leukocytes.[5]

In the present experiment male rats were subjected to a series of one or more doses of 75 r gamma radiation at seven day intervals between exposures. Hematological examinations were made following the last exposure in each series of irradiations. This experimental design was selected in order to determine the effects of periodic exposure to sublethal doses of low intensity ionizing radiations upon recovery in the hematopoietic system as manifested by changes in the circulating blood cells. Presumably, observable effects would be the resultant of interaction between radiation damage caused by the periodic exposures and the forces of regeneration active during the interval.

EXPERIMENTAL DESIGN AND METHOD

Four hundred and thirty-two adult male Sprague-Dawley rats bred in the Laboratory colony were employed in this study. They were given food (Purina chow) and water ad lib. One half of the rats was distributed into six groups receiving different numbers of periodic exposures to gamma rays (75 r, 2 x 75 r, 3 x 75 r, 4 x 75 r, 6 x 75 r, and 8 x 75 r). These groups were divided further with respect to the six days of sacrifice (days 1, 5, 8, 14, 23, and 34), making a total of 36 irradiated groups. The remaining half of the animals comprised another set of 36 sham-irradiated groups which served as controls for the effects of radiation.

To facilitate experimental procedures and to control environmental variations, the total experimental pattern was repeated six times with one representative of each group for each replication. In each replication all animals were from litters born during the same week and were of similar body weight.

Animals were placed in small Lucite containers for irradiation and exposed to an isodose gamma radiation field (9.4 r/hr) for eight hours resulting in an air dose of 75 r for each irradiation. The radiation factors were: cobalt-60, approximately 1.3 Mev, 8.7 curies, point source. The controls were placed in the radiation exposure chambers and sham-irradiated.

On the days of sacrifice cardiac blood was drawn for the determination of the hematocrit, hemoglobin, and the erythrocyte, leukocyte and reticulocyte estimations. Standard Wright Stain blood smears were used for differential leukocyte counts. The mean corpuscular hemoglobin (M.C.H.), the mean corpuscular volume (M.C.V.), and the mean corpuscular hemoglobin concentration (M.C.H.C.), as listed in Table 1, are quotients for the ratios of hemoglobin/RBC count, hematocrit/RBC count, and hemoglobin/hematocrit, respectively.

Since each of the six irradiated animals in each group was matched with its own sham-irradiated control, the statistical analysis was based on differences between paired animals for each group. The probability tables for individual comparisons by the ranking methods of Wilcoxon[8] were utilized for statistical evaluation of the data. Significant changes were referable to the probability level of $p \leq 0.05$.

RESULTS

A single exposure to 75 r low intensity gamma radiation during an eight hour period caused a significant decrease in the erythrocyte count of all groups by day 14 post radiation (Fig. 1). At that time a decrease of approximately 2 x 10^6 cells was observed. The erythrocyte count of rats receiving more than one dose of 75 r was always lower than that of the controls; however, the maximum depression was similar for all groups during the post-irradiation period regardless of the number of previous exposures.

Hemoglobin and hematocrit changes were in a similar direction to that of the erythrocyte count (Figs. 2, 3). The mean corpuscular hemoglobin value was larger for the irradiated rats on nearly all days of sacrifice regardless of the number of periodic exposures (Table 1). The maximum increase occurred on day 14 which was at the time of the lowest erythrocyte counts. The mean corpuscular volume of the irradiated rats was also larger than that of their respective controls (Table 1). The points of significant differences were nearly identical to those for the M.C.H. (Table 1). The mean corpuscular hemoglobin concentration was approximately 29 per cent for all groups and remained the same throughout the study (Table 1). There was no significant difference between irradiated rats and their controls.

The values for the reticulated erythrocytes of the exposed rats were low for the early days following the last exposure for each series of irradiations (Fig. 4). A recovery towards control counts was observed thereafter.

A decrease in the number of leukocytes was apparent on day one post irradiation (Fig. 5). The decrease was of similar magnitude for all irradiated groups regardless of the number of previous exposures. Recovery was initiated by day 5, and continued until day 34, at which point the values obtained from the irradiated rats were either normal or approaching it. The recovery in leukocyte counts was slightly more retarded in groups exposed more than once.

As can be observed from Fig. 5, the decrease in the total number of leukocytes was mainly due to a decline in lymphocytes. The neutrophil values obtained from irradiated rats were also always smaller than that of their controls (Fig. 6); however, their decline was less than that of the lymphocytes.

DISCUSSION

The anemia observed in animals irradiated with massive doses is a result of decreased hematopoiesis[11] and of a lowered capillary resistance which permits the escape of erythrocytes into the lymphatic system.[14] However, the latter does not occur in animals irradiated with sublethal doses, as was demonstrated in recent experiments with rabbits.[13] Presumably, changes in the number of erythrocytes in the rats of the present experiment which were subjected to low intensity radiation were caused by damage to the erythropoietic tissues. Such damage at low doses was observed by Bloom,[10] who found that a dose of 80 r of X rays caused acute injury to the bone marrow as indicated by dead erythroblasts and increased nuclear debris. Indirect evidence was obtained by Hennessy and Huff.[12] They noticed that the iron uptake of rats subjected to 125 r of X rays was decreased about 40 per cent from the control values within the first five days post-irradiation. These authors stated that they were able to detect statistically significant depressions in tracer ion uptake in animals exposed to 5 and 25 r of X rays.

In the present experiment, the anemia of the irradiated rats was distinct, albeit slight and macrocytic. The cause of the macrocytic anemia is now known. Stearner, et al,[5] observed this type of anemia in irradiated rats and Wintrobe[9] reports such occurrences in human dial workers.

The outstanding result of this study was the extent of recovery apparently possible in the erythropoietic system during the seven day interval between periodic exposure to low intensity gamma irradiation, regardless of the number of previous exposures. Although differences attributable to previous periodic irradiations were observed one day after the last exposure, the extent of maximum red cell depression observed for all groups was similar and it occurred at about the same time (day 14). The latter fact suggests that the erythrocyte decline was, to a great extent, reflective of only the last exposure.

The remarkable ability for regeneration of the erythroid system has been observed in recent experiments. Bloom[10] noticed in histological studies that erythroblasts, in spite of their great sensitivity to irradiation, were the first cells to regenerate. Valentine and Pearce[15] irradiated (200 r, X rays) phlebotomized cats having a 40 per cent erythrocyte decrease and found the recovery from phlebotomy was only slightly inhibited. This procedure was repeated four times on six cats[16] at four to six month intervals, and little evidence was obtained of any permanent damage to the erythropoietic system of these cats. The authors emphasized the fact that a dose of 200 r caused a profound insult to the hematological structure of cats, however, complete functional recovery occurred in the intervals between radiation exposures. It appears plausible that the relatively radioresistant stem cells of the marrow give first preference to the restoration of the impaired erythrocyte system.

Erythropenia in the present study was preceded by a slight but significant reticulocytopenia. The latter returned to normal values after eight days. Ingram, et al.,[3] reported such decreases in the reticulated cells of rats for single X-ray doses of 50 r and higher with recovery occurring approximately nine days after irradiation.

The early appearance of leukopenia in the present experiment was due to the short life span of leukocytes, primarily the lymphocytes. Inadequate production of new cells because of damaged myeloid and lymphoid precursors, therefore, will be observed within hours in the circulating blood. No additive destructive effect of previous exposures was detected, and in general recovery was good. However, regeneration was somewhat retarded in groups subjected to more than two doses of 75 r gamma radiation. This may indicate some residual influence upon recovery processes of the leukocyte system. It is of interest to note here that Valentine and Pearce[15] observed a slower recovery of leukocytes in irradiated phlebotomized cats.

The results observed in the present study suggest that only slight, if any, cumulative effects are detectable in peripheral blood elements during the first 34 days after several (2-8) weekly exposures to low intensity gamma radiation. This is probably indicative of the recovery capacity of the hematopoietic tissues during the seven day interval between exposures.

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TABLE 1

CALCULATED VALUES OF CELLULAR VOLUME, HEMOGLOBIN AND HEMOGLOBIN CONCENTRATION

	Dose (r)	Group	Days of Sacrifice Post Irradiation 1	5	8	14	23	34
	75	C[1]	64.0	59.1	67.5	64.5	70.3	67.5
		I[2]	65.6	<u>75.9</u>	70.7	<u>84.9</u>	76.4	78.9
Mean	2x75	C	63.5	66.3	69.7	56.9	61.7	68.7
Corpuscular		I	74.3	76.9	72.3	<u>85.6</u>	<u>79.1</u>	72.1
Volume	3x75	C	68.9	73.2	66.6	63.4	63.4	68.7
		I	74.7	<u>82.7</u>	75.5	<u>83.7</u>	<u>83.4</u>	82.7
cubic	4x75	C	63.1	68.4	63.4	61.2	60.9	64.1
microns		I	73.1	76.2	<u>82.9</u>	<u>85.2</u>	<u>76.3</u>	68.4
	6x75	C	71.3	69.9	70.7	71.6	67.9	71.4
		I	71.5	<u>82.6</u>	74.9	<u>85.3</u>	74.5	68.7
	8x75	C	66.8	75.4	65.8	64.4	67.3	67.7
		I	<u>75.2</u>	80.8	<u>83.1</u>	<u>88.9</u>	<u>78.2</u>	<u>78.9</u>
	75	C	19.2	17.6	20.1	18.8	20.2	19.4
		I	19.2	<u>23.3</u>	20.9	<u>26.2</u>	22.7	24.4
Mean	2x75	C	19.0	19.5	20.1	18.4	18.1	20.0
Corpuscular		I	22.0	23.2	21.6	<u>25.3</u>	22.4	20.1
Hemoglobin	3x75	C	19.8	21.4	19.4	18.5	19.2	19.8
		I	22.4	<u>24.0</u>	22.3	<u>25.3</u>	<u>23.5</u>	23.5
micro	4x75	C	18.1	19.7	18.2	19.6	18.0	18.0
micrograms		I	20.1	22.0	<u>23.9</u>	<u>24.5</u>	<u>21.7</u>	19.5
	6x75	C	19.9	19.6	20.2	21.4	19.7	20.6
		I	21.1	<u>25.6</u>	21.8	<u>25.0</u>	<u>22.8</u>	19.6
	8x75	C	18.9	21.4	18.4	19.7	19.6	19.4
		I	<u>22.1</u>	24.2	<u>25.7</u>	<u>26.7</u>	<u>22.9</u>	23.4
	75	C	29.9	29.9	29.9	29.2	28.8	28.7
		I	29.5	30.6	29.7	31.5	29.7	30.8
Mean	2x75	C	30.0	29.4	29.6	30.3	29.4	29.1
Corpuscular		I	29.7	30.0	30.0	29.5	28.5	28.9
Hemoglobin	3x75	C	28.8	29.2	29.2	29.2	30.4	28.8
Concentration		I	30.8	29.1	29.5	30.3	28.1	28.6
per cent	4x75	C	28.8	28.8	28.6	30.6	30.1	28.1
		I	28.3	28.9	28.6	28.8	28.4	30.0
	6x75	C	28.0	28.0	28.2	29.9	28.8	28.8
		I	29.9	32.0	29.1	30.5	30.7	28.6
	8x75	C	28.3	28.3	28.0	28.9	29.2	28.7
		I	29.3	30.1	31.0	30.1	29.5	29.1

1. C = Controls
2. I = Irradiated Rats
3. Underlined mean values of pairs differ significantly, $P \leq 0.05$.

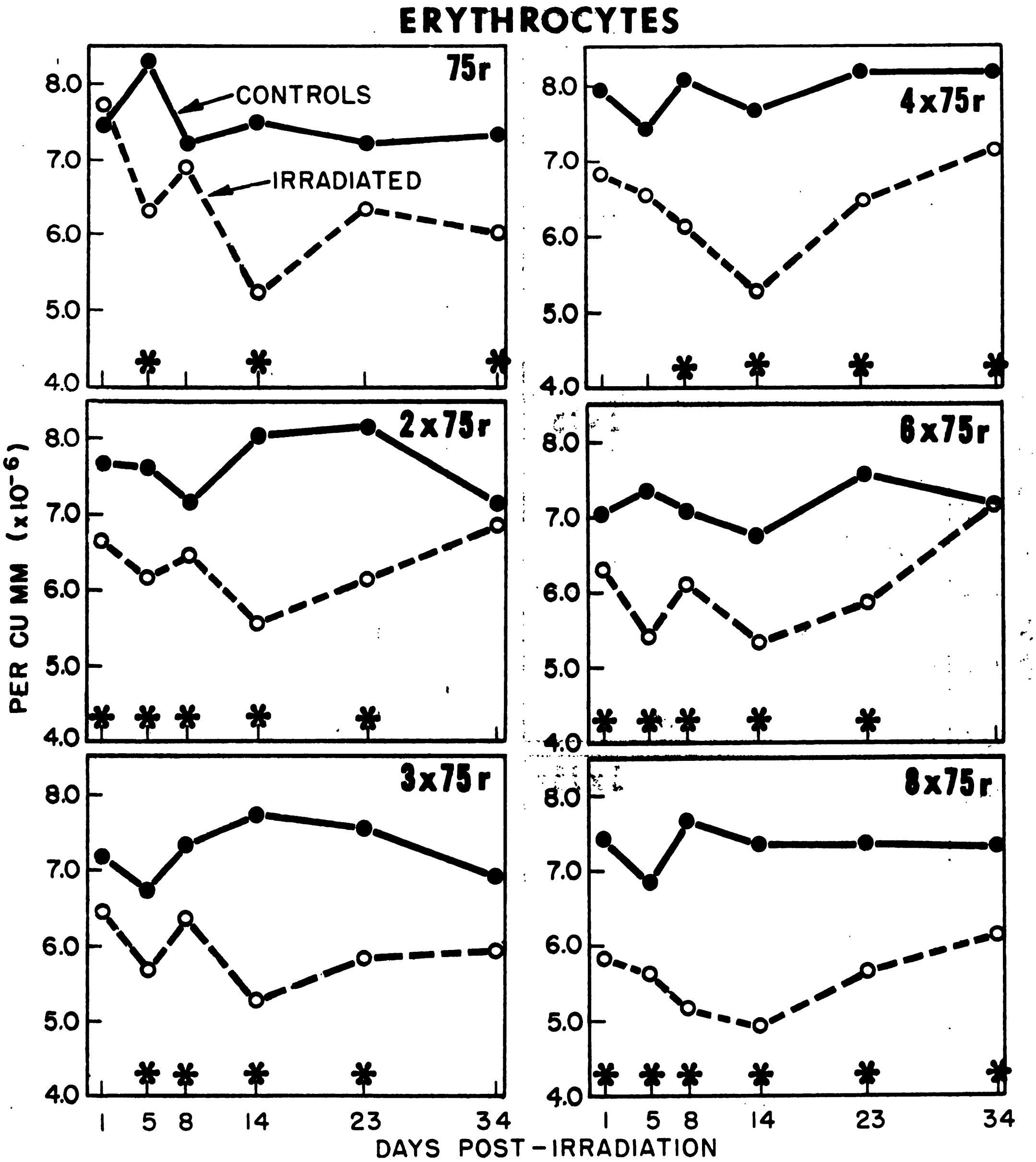

Fig. 1 Erythrocyte Values of Rats Subjected to Various Numbers of 75 r Doses of Gamma Radiation. Significant (5 per cent level) differences between mean values are indicated by asterisks.

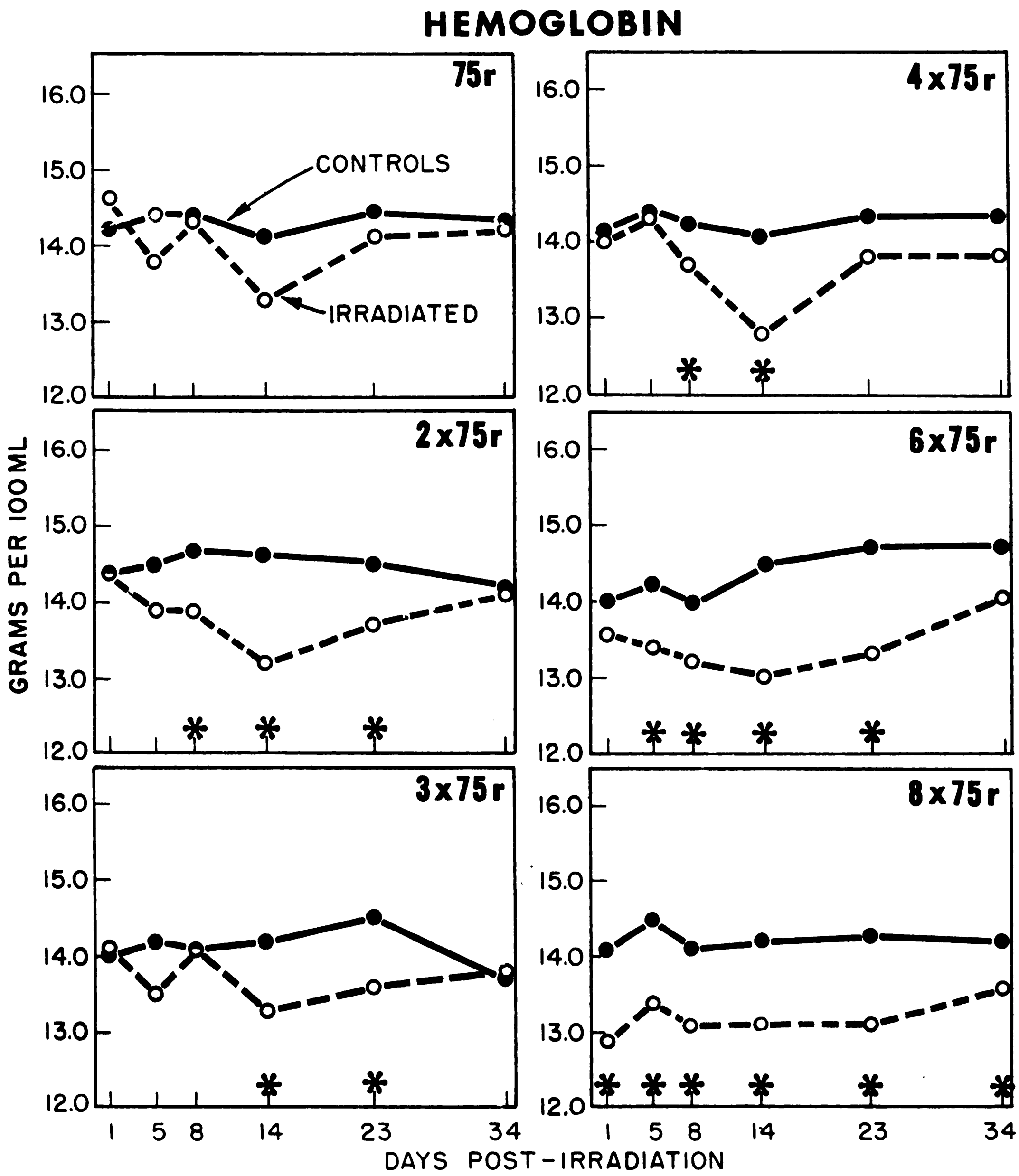

Fig. 2 Hemoglobin Values of Rats Subjected to Various Numbers of 75 r Doses of Gamma Radiation. Significant (5 per cent level) differences between mean values are indicated by asterisks.

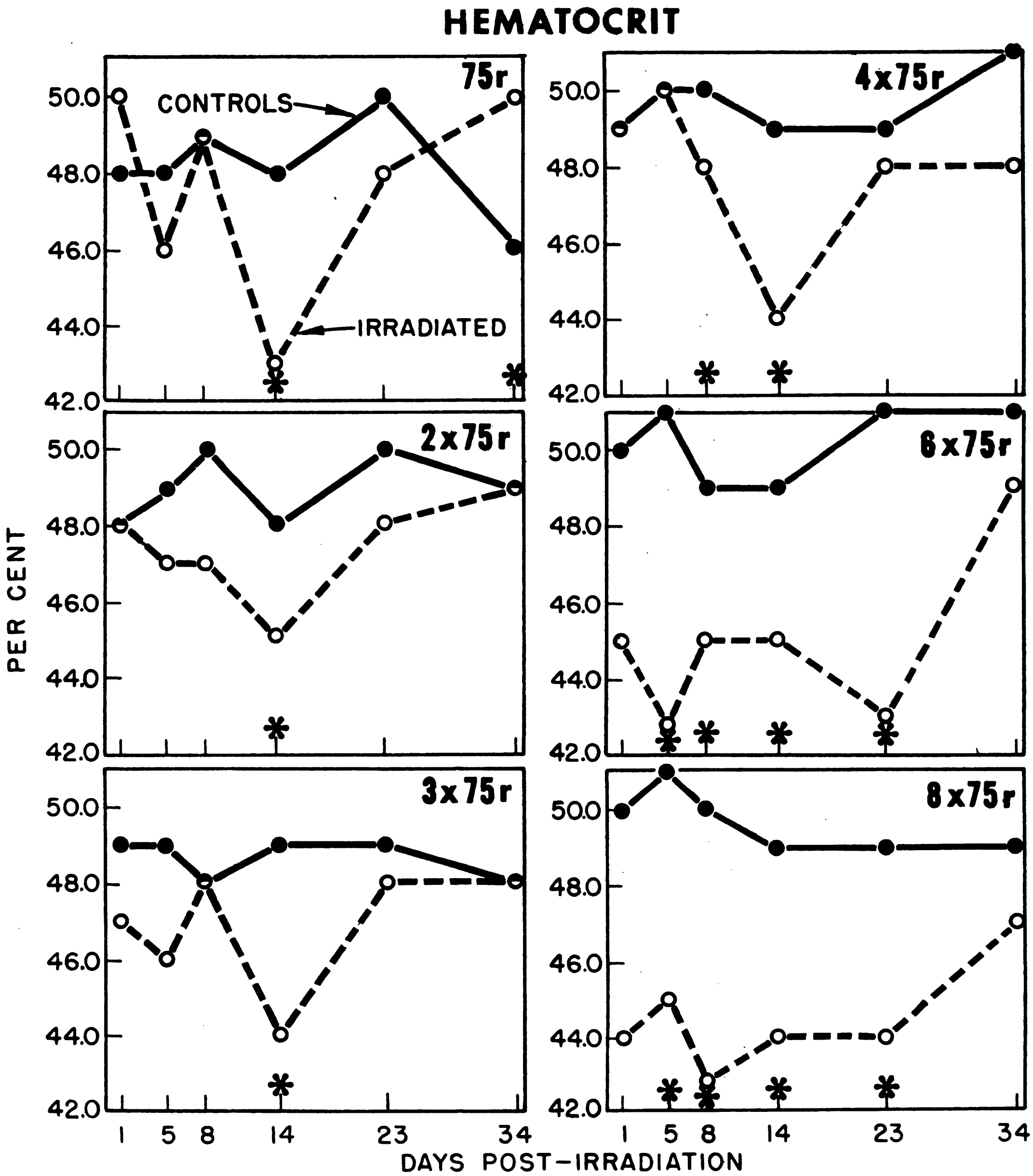

Fig. 3 Hematocrit Values of Rats Subjected to Various Numbers of 75 r Doses of Gamma Radiation. Significant (5 per cent level) differences between mean values are indicated by asterisks.

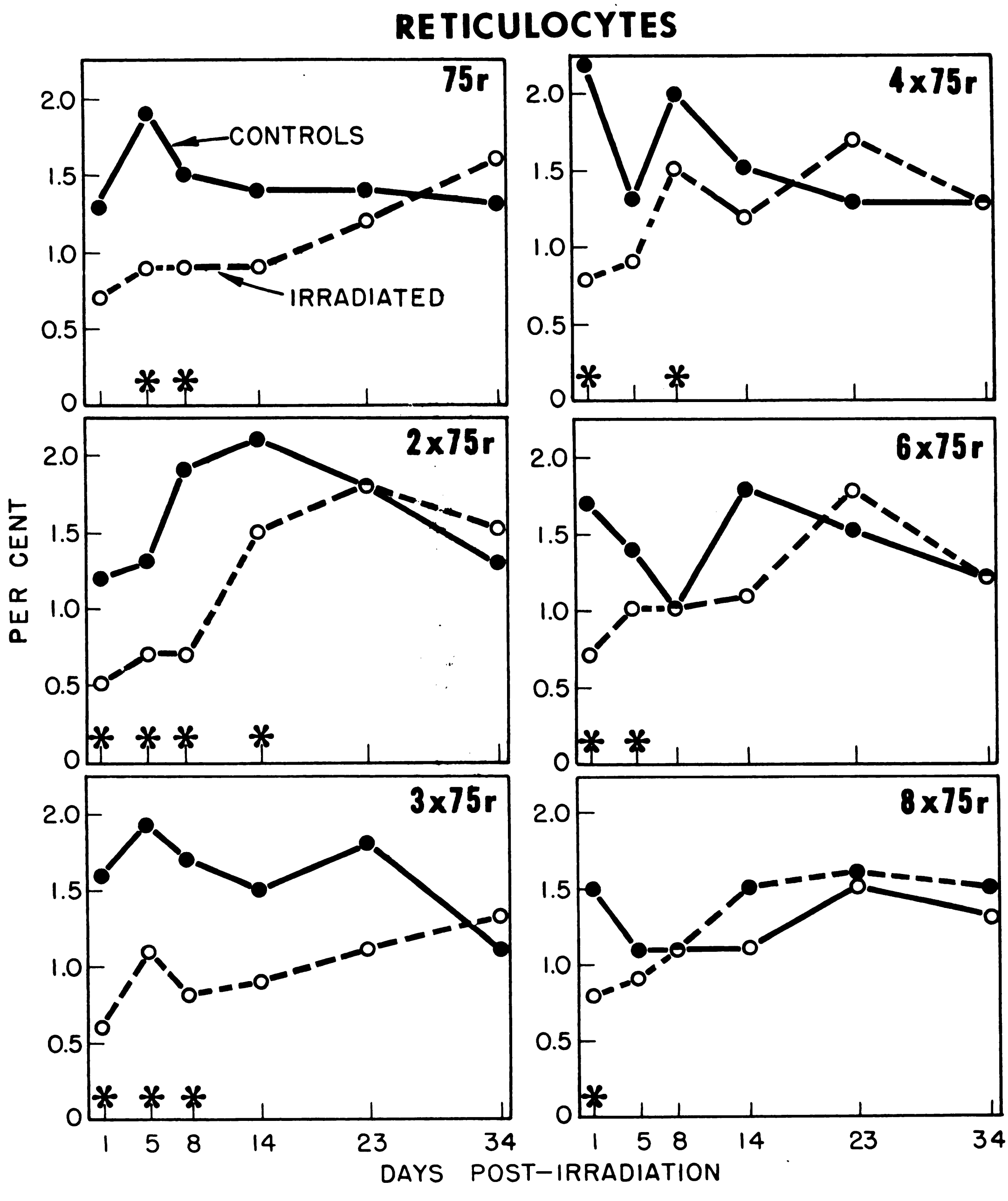

Fig. 4 Reticulocyte Values of Rats Subjected to Various Numbers of 75 r Doses of Gamma Radiation. Significant (5 per cent level) differences between mean values are indicated by asterisks.

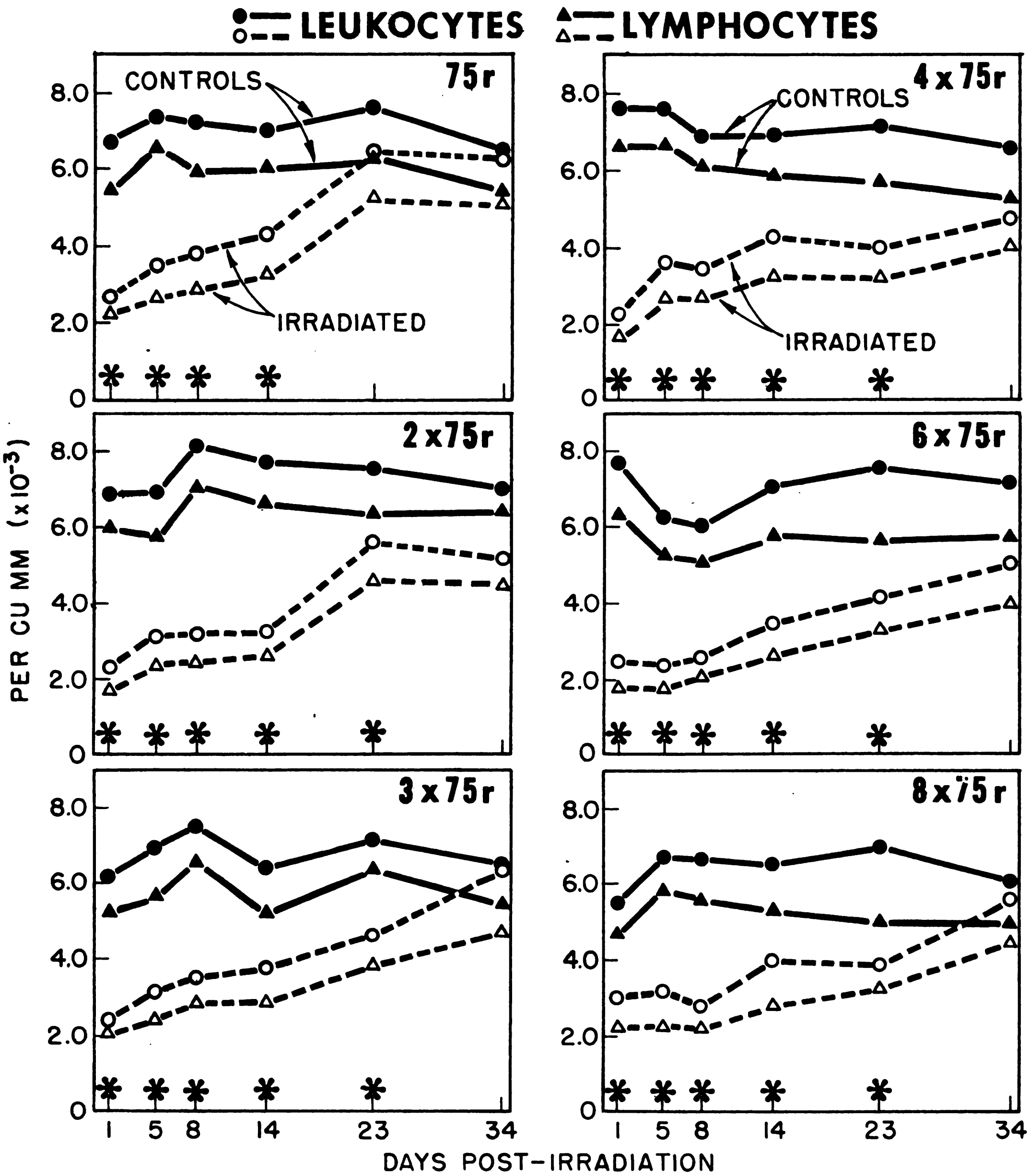

Fig. 5 Leukocyte and Lymphocyte Values of Rats Subjected to Various Numbers of 75 r Doses of Gamma Radiation. Significant (5 per cent level) differences between mean values are indicated by asterisks.

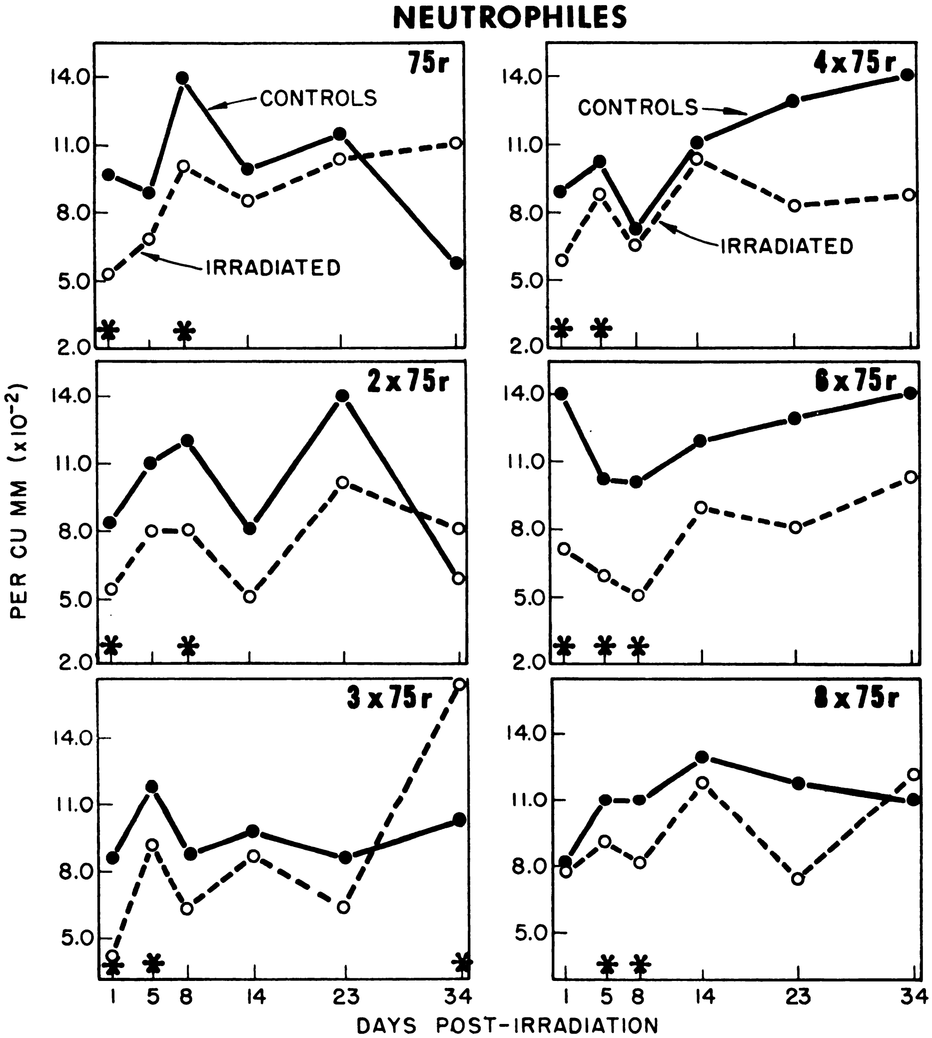

Fig. 6 Neutrophil Values of Rats Subjected to Various Numbers of 75 r Doses of Gamma Radiation. Significant (5 per cent level) differences between mean values are indicated by asterisks.

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EFFECT OF REPETITIVE EXPOSURE TO GAMMA RAYS ON THE HEMATOPOIETIC SYSTEM OF THE RAT, by S.J. Baum, D. J. Kimeldorf, and E.M. Jacobsen. 27 Aug. 1954. iv, 18 p. diagrs. (16 refs.) UNCLASSIFIED

Male rats were exposed one or more times to 75 r gamma at seven day intervals. Hematological examinations after the last exposure indicated that the maximum changes were largely independent of the number of exposures and reflected mostly the effects of the last irradiation. These results are ascribed to the capacity of the hematopoietic system to recover during the seven day intervals.

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2. Blood forming organs - Radiation injuries
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